SECTION DRYING IN LATE-SEASON GRAPEFRUIT

BY

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SECTION DRYING IN LATE-SEASON GRAPEFRUIT

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The occurrence of section drying (resulting from juice-vesicle disorders) and in-fruit germination were studied in two leading grapefruit cultivars (Citrus paradisi Macf. cv. 'Marsh seedless' and 'Ruby Red') grown in 8 groves over 3 consecutive years (1986-1988). Section drying in grapefruit resulted from both vesicle collapse and granulation. However, vesicle collapse was the primary factor contributing to section drying in grapefruit. As the season progressed, there was a progressive increase of section dried fruit as well as fruit containing germinating seeds. Variations in the amount of section dried fruit and in-fruit germination were observed between years, groves, harvest time, and storage duration. During storage, both section drying and in-fruit germination increased greatly. Positive correlations were found each year between the severity of section drying and the percentage of fruit containing

germinating seeds. However, other potential factors were likely involved because only 50% of the total variation in in-fruit germination could be accounted for by section drying.

Much of the work on juice-vesicle disorders resulting in section drying in citrus has focused on granulation. In this study, the physiological and biochemical changes occurring in disordered juice-vesicles (granulation and vesicle collapse [dehydration disorder]) were compared, particularly the changes in cell-wall polysaccharides. It appeared that granulation was associated with an increase in cell wall polymers whereas collapsed vesicles exhibited a modification in wall polymers, especially pectins. The physiological and biochemical differences in juice-vesicle disorders (granulation and vesicle collapse) are discussed.

INTRODUCTION

Late cultivars of citrus have a long harvest period. However, when fruit are harvested late in the season, quality is often adversely affected due to the occurrence of juice-vesicle disorders among other factors. Late-season grapefruit produced in Florida are often afflicted with vesicle disorders which result in drying of the affected fruit segments. Section drying also occurs during storage and/or transit. This can be a serious problem for growers because fruit are often harvested late in the season, at which time profits can be high if the fruit is sound.

Granulation is frequently found in orange and mandarin cultivars grown throughout the world. Among citrus cultivars, grapefruit has been known to be less susceptible to granulation than other types of citrus. Furthermore, visible symptoms of grapefruit vesicle disorders are different from granulation. For these reasons, it has been thought that vesicle disorders occurring in grapefruit might not be related to granulation. Nevertheless, no detailed studies have been carried out to identify the types of vesicle disorders affecting grapefruit.

In-fruit germination is another potential problem in late-season grapefruit. Few studies have been conducted to

estimate the frequency with which fruit are affected by in-fruit germination in Florida grapefruit. Since seed germination within grapefruit is often high in disordered fruit, germination is thought to be somehow associated with vesicle disorders.

The objectives of this study were 1) to identify the type of vesicle disorders occurring in grapefruit, 2) to estimate the extent to which fruit are affected by vesicle disorders and/or in-fruit germination, 3) to examine the relationship between vesicle disorders and in-fruit germination, and 4) to examine the physiological and/or biochemical changes of the vesicles affected by the disorders, particularly in their cell walls.

LITERATURE REVIEW

Introduction

Grapefruit, particularly in Florida, have a relatively long harvest time, usually from October through May. When grapefruit are harvested late in the season, fruit quality is often adversely affected because of the occurrence of disorders affecting juice vesicles. At least two different types of vesicle disorders have been described in citrus: granulation and dehydration (Bartholomew et al., 1941; Noort, 1969; Albrigo et al., 1980). Many studies have focused on understanding and preventing these vesicle disorders but no successful methods have been found. Although the two vesicle disorders exhibit quite different visible symptoms, no detailed comparative studies have been undertaken.

In addition to vesicle disorders, in-fruit germination is a potential problem in late-season grapefruit harvested directly from the tree or during postharvest storage because even 'seedless' cultivars contain viable seeds which may germinate (Albrigo and Carter, 1978). Fruit containing germinating seeds exhibit off-flavors and are not desirable

for either fresh market or processing. The Florida Dept. of Citrus (1975) states that grapefruit cannot be extracted for juice if excessive sprouted seeds are present. Neither the amount of fruit effected by in-fruit germination nor the potential factors associated with seed germination within fruit have been adequately studied.

Physiological Changes in Fruit during the Development of Vesicle Disorders

Granulation (G) is a well known juice-vesicle disorder in citrus. Symptoms of G include hardening of vesicles, uneven cell-wall thickening in juice-cells, lignification and, at the late stages, a gradual collapse of internal juice-cells (Bartholomew et al., 1941; Matsumoto, 1964). Increases in dry weight, ethenol-insoluble solids, minerals (K^+, Na^+, Ca^{2+}, Mg^{2+}, and PO_4), and pectic substances (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961) are also characteristic of G. Several studies have demonstrated a slight but significant decrease in water-soluble-pectin (WSP) in juice from granulated 'Valencia' oranges (Gilfillan and Stevenson, 1977; El-Zaftawi, 1978). Others have shown that juice-vesicles or juice from granulated fruit contain more total pectins than healthy vesicles or fruit (Sinclair and Jolliffe, 1961; El-Zaftawi, 1978). This seems to indicate that pectins in granulated tissue exist as water-insoluble forms rather than water-soluble pectic acids. High methoxy-content of ethanol-insoluble-solids (EIS) from

granulated vesicles (Sinclair and Jolliffe, 1961) and low pectinmethylesterase (PME) (E.C. 3.1.1.11) activity in granulated fruit (Chakrawar and Singh, 1977b) seem to support this speculation. Hardening of the juice vesicles during G was suggested to result from gelation caused by increased levels of pectin and divalent cations, especially calcium (Sinclair and Jolliffe, 1961). Since Ca^{+2} binding to poly-D-galacturonate contributes to the stabilization of pectic polysaccharides (Morries et al., 1982; Selvendran, 1985), this might be a partial explanation of low WSP in granulated vesicles. The observation that WSP decreased while total pectin increased indicates that there should be an increase in other pectin fractions (e.g., chelator- or alkali-soluble pectins). On the contrary, in a comparative study of juice components extracted from granulated and non-granulated 'Valencia' and navel oranges, Gilfillan and Stevenson (1977) found a decrease of not only WSP but also chelator- and alkali-soluble pectins in granulated navel fruit juice but not consistant in 'Valencia' fruit juice. However, pectins in extracted juice may not be representative of the pectins in cell walls.

In addition to pectin changes, the presence of lignin components in granulated vesicles has been reported (Bartholomew et al., 1941). Lignin is a complex, 3-dimensional cell-wall polymer present in vascular plants and usually associated with vascular tissue (Eskin, 1979). In pear fruits, lignin exists as a component of a

lignocellulose complex in mesocarp stone cells (Eskin, 1979). Since lignification strengthens the cell walls, hardening of granulated vesicles may be associated with lignin synthesis. No quantitative analyses have been performed to test this possibility. A previous study of lignin was confined to histochemical detection (Bartholomew et al., 1941). Data on the detection of lignin in granulated vesicles (Bartholomew et al., 1941) and on the increase of pectin (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961) indicate that alterations in cell-wall synthesis and/or qualitative changes in cell-wall polysaccharides could be a potential factor contributing to granulation.

Studies on the relationship between mineral content and vesicle disorders have provided inconsistent data. Sinclair and Jolliffe (1961) found that calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), and phosphorous (P) increased in granulated juice-vesicles whereas Gilfillan and Stevenson (1977) reported that no consistent increase in minerals in juice from granulated fruit occurred. These inconsistencies may possibly be explained by the sampling methods. Sinclair and Jolliffe (1961) examined the total mineral contents from isolated juice vesicles (normal or granulated) whereas Gilfillan and Stevenson (1977) examined the mineral content of filtered juice from normal or granulated fruit. Since the filtered juice does not contain total insoluble solids, it may not represent the composition of cell wall minerals.

Boron (B) in extracted juice showed a negative correlation to G in naval but not in 'Valencia' oranges (Gilfillan and Stevenson, 1977). The exact physiological role of B in planta remains unclear. A major portion of B is associated with membranes (Tanada, 1983) and it may contribute to the maintenance of membrane integrity alone or with calcium (Pollard et al., 1977, Pilbeam and Kirkby, 1983; Lovatt and Dugger, 1987). Boron also has been known to be related to the cell-wall synthesis. Since under B deficient condition, cell-wall thickening in sunflower root was reported (Hirch and Torrey, 1980). An increase of the non-celluloaic cell-wall polymers under B deficiency has been observed in cotton ovules cultured in vitro (Dugger and Palser, 1985) and in leaves of oil palm plant (Rajaratnam and Lowey, 1974). Thus, data in the above observations indicate a potential association of boron deficiency with the increased of pectin contents in granulated fruit. However, trials to prevent G through spraying boric acid on whole citrus plants showed inconsistant results. Under South African conditions, a boric acid spray was ineffective on oranges (Ann. Rep. Res. Hort. South Africa, 1978-79) whereas in India, it was effective on mandarins (Singh and Singh, 1981a). Whether or not these inconsistant results were caribed from the differance of spraying time is not known. In leaf analysis of sweet oranges, Munshi et al. (1978) found differences in nutriant content between leaves from shoots bearing more G and less G fruit. Nitrate (N),

P, and K^+ increased whereas Ca^{2+}, B, and Zinc (Zn^{2+}) decreased in the shoots with granulated fruit. Interestingly, Ca^{2+} was higher in granulated vesicles (Sinclair and Jolliffe, 1961) but shoots bearing granulated fruit contained less Ca^{2+}. The authors suggested that Ca^{2+} might exist in a bound form (e.g., calcium pectate) in granulated vesicle decreasing Ca^{2+} availability to other vesicles. On the basis of citrus fruit anatomy, this interpretation does not appear logical. Granulation usually begins at the stem-end, but juice-vesicles are individually connected through long hair-like stalks to the vascular bundles located around the septal membrane (Schneider, 1968). Thus, mineral movement may occur from vascular bundles through stalks (symplastic connection), not through interspaces between vesicles (apoplast).

Since N in general induces vigorous growth of plants, it is reasonable to speculate that high levels of N in the shoot are associated with the incidence of G. Granulation is more pronounced on vigorously growing plants (Bartholomew et al., 1941). Also, potassium increases fruit size in citrus (Embleton et al., 1956) and thus high levels of K^+ in shoots may induce G through increasing fruit size.

Zinc deficiency was proposed to be a potential factor contributing to G through affecting auxin level (Awasthi and Nauriyal, 1972; Singh and Singh, 1981a). Indole-3-acetic acid (IAA) synthesis and turn-over may be affected by Zn^{2+} (Mengel and Kirkby, 1982). Tryptophan (a precursor of IAA)

synthesis is reduced under Zn^{2+} deficiency. Also, peroxidase, which is known to be associated with the breakdown of IAA, increases under Zn^{2+} deficiency (Mengel and Kirkby, 1982). Singh and Singh (1981b) also observed that a nutrient spray on the whole plant was effective at reducing G. Effective nutrients included a combined treatment of Zn^{2+}, Cu^{2+}, Ca^{2+}, S, and K^{+}. These results indicate that if G is associated with whole-plant nutrient condition, then interactions between elements may be more important than an effect of any single element.

Granulation reduces fruit quality because of the reduction of extractable juice (Bartholomew et al., 1941; Matsumoto, 1964; Awasthi and Nauriyal, 1972; El-Zeftawi, 1973), lower acid (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961; Awasthi and Nauriyal, 1972; El-Zeftawi, 1973; Chakrawar and Singh, 1977b), lower total-soluble-solids (TSS) (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961; Gilfillan and Stevenson, 1977; Chakrawar and Singh, 1977b; El-Zeftawi, 1978), and lower total and reducing sugars (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961; Matsumoto, 1964). A low level of sugars and acids is closely related to G (Bartholomew et al., 1941; Gilfillan and Stevenson, 1977; El-Zeftawi, 1978; Ann. Rep. Rec. Hort. 1978-1979). Low sugars and acids in the stem end and in large fruit (Ting, 1969) seem to be consistent with the prevalent occurrence of G in the stem-end or in large fruit. However, fruit produced inside the tree canopy or on

the northern aids of treas which tend to contain high acid levels (Syvertsen and Albrigo, 1980), are still susceptible to G (Bartholomaw at al., 1941; Awasthi and Naurıyal, 1972). Gilfillan and Stevenson (1977) found that TSS, acids, and WSP, in that order, showed a weak but consistent negative correlation with G. Whether or not the decreaas of sugars and acids is a primary factor or secondary symptom of G is not clearly understood.

Citrus fruit quality may also be reduced by juice-vesicle dehydration, a disorder which has baan reported in oranges and mandarins. Dry juice sac (stylar-end granulation) in 'Valencia' orangas in California usually occurs at the stylar end of small fruit late in the saason or postharvest (Bartholomew et al., 1941), whereas in South Africa core dryness in navel orangas is often found in the central fruit portion of large fruit late in the saason or during transit (Noort, 1969). Section drying (SD), prisarily dua to vesicle collapse (VC), was reported in late season grapefruit grown in Florida (Albrigo et al., 1980).

Common symptoms of dehydration disorders include a gradual shrinkage and complete collapse of the affected vesicles (Bartholomew et al., 1941; Matsumoto, 1964; Noort, 1969). Available information is limited for this dehydration disorder in grapefruit. Although symptoms are quite different fros those exhibited by G (for example, no hardening of disordered vesicles), increased lignin content was demonstrated in partially collapsed vesicles in

grapefruit (Burns and Achor, 1988). The WSP decrease in juice derived from the stem-end portions of both G and core-dried fruit has been reported. however, the measurements of WSP in core-dried fruit were not carried out in the fruit affected by core-dryness alone since the fruit also were affected by G (Ann. Rep. Res. Hort. South Africa, 1978-1979). A transient increase in ethylene production in some mandarin types occurs prior to the fruit being affected by dry juice sac (Sinclair, 1984). Watermelons, which exhibit low levels of polygalacturonase (PG, E.C. 3.2.1.15), are adversely affected by ethylene treatment and develop a tissue water-soaking which results from degradation of the middle lamella (Elkashif and Huber, 1988). However, whether VC in grapefruit is induced by the same mechanism is not known. Citrus is a typical non-climacteric fruit which is usually less sensitive to ethylene (Rhodes, 1980). Dry juice sac in oranges was suggested to result from the excess water loss by the leaves when insufficient water was present in the soil (Sinclair, 1984). In this scenario, water loss below a certain critical point does not allow recovery of vesicles to the original turgidity and they remain desiccated. Since water movement from vesicles once stored is restricted in citrus fruit (Kaufman, 1970), vesicle dehydration may occur only under severe drought condition. No direct evidence has been provided for this speculation that water loss is a prime factor responsible for dry juice sac during growing season. In grapefruit, VC occurs only in

tha lata saaaon or during storaga. Also, fruit wrappad individually in polyethylene film, which greatly reducas water losa in fruit aftar harvest, did not reduca auction drying in grapefruit stored at 13 °C or higher (Albrigo at al., 1981). Thia indicates that water loss may not be a primary factor associated with thia vesicle disorder in grapefruit.

Soma similaritias exist batwaen G and dahydration disorders. These include low levels of WSP, sugars, acids, and tha apparant praaanca of lignin componants. However, no cooparative studies have been coapleted. Thus, part of thia study focused on comparing G and VC of grapefruit juice vesicles.

Potential Factors Associated with Juice-Vesicle Disorders

Various factors have been reported to be associated with G. Thase includa rootstock, fruit aiza (Bartholomew at al., 1941; Awasthi and Nauriyal, 1972; El-Zaftawi, 1978), cultivar (Awasthi and Nauriyal, 1972), vigor and/or age of plant (Bartholomew et al., 1941; Awasthi and Nauriyal, 1972), climate (Bartholomew et al., 1941; Noort, 1969; Awasthi and Nauriyal, 1972; El-Zaftawi, 1978), saaaon (Bartholomew at al., 1941; Chakrawar and Singh, 1977), cultural practicas (Bartholomaw at al., 1941; Bartholomaw and Sinclair, 1944; Hield and Erickson, 1962), and storage temperature (El-Zaftawi, 1976). In ganaral, large fruit

from vigorously growing trees show a higher incidence of G (Bartholomew et al., 1941; Awasthi and Nauriyal, 1972). However, no key factor(s) has been consistently found. Attempts to control G through plant growth regulator application have yielded inconsistent results. Singh and Singh (1981b) reported that spray of GA_3 or planofix (1-naphthalene acetic acid, NAA) reduced G in a mandarin cultivar. In other studies, a preharvest spray of 2,4-dichlorophenoxyacetic acid (2,4-D) was effective to delay the incidence of G in 'Valencia' oranges but no further effect was observed once G initiated (Hield and Erickson, 1962) or was ineffective on oranges produced in South Africa (Ann. Rep. Res. Hort. South Africa, 1978-79). Planofix treatment also reduces G in oranges through a correction of hormonal imbalance (Singh and Singh, 1981b); however, the effect of auxin on G may be necessary to be reexamined because of the inconsistent results.

Singh and Singh (1981b) reported that preharvest treatment with 2-(chloroethyl) phosphonic acid (ethrel) significantly reduced the incidence of G on mandarins. They suggested that ethrel enables fruit to avoid G by ripening the fruit earlier.

The fact that dehydration disorders (dry juice-sac and core dryness) occur only in the late season or during postharvest storage or transit (Bartholomew et al., 1941; Noort, 1969) indicates a potential relationship between the disorders and fruit senescence. Noort (1969) found a

tendency for the amount of dehydration disorders and G to be greater if there were higher winter temperatures before new growth of plant. Bartholomew et al. (1941) observed a similar result in that the frequency of dry juice-sac was more pronounced following a relatively warm winter SD in grapefruit rarely occur in the early season but become severe during transit or storage (Albrigo et al., 1980, 1981).

Cell-Wall Alteration during Fruit Maturation and Senescence

Dehydration disorders in citrus result in the complete collapse of juice vesicles whereas vesicles affected by G usually maintain their original shape prior to the final stage when some shrinkage occurs (Bartholomew et al., 1941; Bartholomew and Sinclair, 1944). This indicates that abnormal cell-wall degradation and/or synthesis at the late stage of fruit development might be associated with these vesicle disorders. Little is known about cell-wall changes in citrus during fruit development and senescence.

The plant cell wall is a specialized form of extracellular matrix surrounding the protoplast of the cell. The composition of plant cell walls is extremely heterogeneous. In general, cell wall components are classified into several groups on the basis of their solubility in various agents. Celluloses, pectins, hemicelluloses, and glycoproteins are the major components

of primary cell walls (Selvendran, 1985; Darvill et al., 1980) although they may differ both quantitatively and qualitatively between species.

Cell walls are dynamic; their shape, composition, and properties are constantly changing in response to growth (Labavitch, 1981; Ray, 1987), the stage of differentiation (Pressey et al., 1971; Grose and Wallner, 1979; Ahmed and Labavitch, 1980; Labavitch, 1981; Roe and Bruemmer, 1981; Huber, 1983b; Grose and Same, 1984) and environmental effects (Sakurai et al., 1987). Textural modifications of fruit tissue, mainly softening, are most evident during fruit ripening. Softening is associated with an increase in solubility as wall as hydrolysis of cell-wall polysaccharides resulting in the loss of intercellular cohesion (Pressey et al., 1971; Brady, 1986). The middle lamella, which is rich in pectin, is believed to be the region of the cell wall intensively affected during fruit softening. Polygalacturonase (PG) is known to be associated with fruit softening in many fruit types (Pressey et al., 1971; Pressey and Avants, 1982; Roe and Bruemmer, 1981; Brady, 1986). Ultrastructural studies show a correlation between the appearance of pectin hydrolyzing activity (e.g., polygalacturonase) and a loss of electron density in the middle lamella region in avocado (Platt-Aloia et al., 1980), pear (Ben-Arie and Kislev, 1979), and tomato (Crookes and Grierson, 1983). During ripening in tomatoes, pectin solubility increases in association with PG-mediated

increaaa in low-molacular waight pactina and decreaaa in high-molecular weight pectins (Huber, 1986). Howaver, an increaaa in pectin aolubility ia not sufficiant evidence for tha praaanca of hydrolytic anzymaa bacauaa pactin aolubilization can occur through a changa in pH or in an altaration in divalent catiana in fruit call walla (Huber, 1983a). Evidance for non-anzymic increasea in pactin solubility can be found in atrawberries (Huber, 1984) and muskmalona (McCollum, 1987), which, whila containing no PG, ahow incraaaaa in aolubla pactin aa fruit ripan.

Citrua ia known to hava only axo-PG (Riov, 1974, 1975), the activity of which ia measurabla only when "uronic acid oxidaaa," namad by Riov (1975), ia inhibited. Grapafruit paal tiaaua braakdown occurs when infactad by pathoganic fungi, tha avants of which ara ralatad to tha accumulation of fraa D-gaiacturonic acid ralaased by fungal exo-PG (Achiiaa at al., 1985). Whathar or not tha accumulation of fraa gaiacturonic acid aa a result of the axo-PG ia involvad with VC in aenaacant fruit ia not known, ainca PG activity ia extremaly low in grapafruit (Riov, 1974). Tha lack of endo-PG in citrua aiao indicatea that modification of wall polysaccharidaa in citrua, if any, muat be mediatad by nonenzymatic machaniams or othar hydrolaaaa (a.g., hamicallulaaaa). No diract avidanca is availabla regarding tha altaration of call wall polymars during grapefruit maturation and aanaacanca.

Studies on the changes in non-cellulosic neutral sugars in various fruit types revealed a net loss of wall sugars during ripening (Gross and Sams, 1984). Information about hemicellulose modification during fruit ripening is available for only a limited number of fruit types. In tomatoes, hemicelluloses exhibit a decrease in high-molecular weight polymers and increases in low-molecular weight polymers during ripening (Huber, 1983b). Hemicellulose changes occur independently from fruit growth and are temporally related to ripening. Strawberries exhibited a similar change in hemicelluloses during ripening (Huber, 1984). The author suggested that the structural modification of hemicellulose is an enzymic alteration; however, enzymes responsible for the modification of hemicelluloses were not investigated. Several glycosidases (primarily β-galactosidase) were proposed to be related to fruit softening in tomato (Wallner and Walker, 1975; Pharr et al., 1976; Pressey, 1983; Watkins et al., 1988) and in apple (Wallner, 1978) fruit. Although these enzymes sometimes appear coincidently with fruit softening, their role in wall degradation is still questionable. Glycosidase activity is assayed with p-nitrophenol-β-glycoside substrate. Since dimers and trimers are the preferable substrate for glycosidases (Reese, 1977), enzyme activity toward p-nitrophenol-β-glycoside is not sufficient evidence that glycosidases can digest high-molecular-weight polymers. Glycosidases thus may require endo-hydrolase activity to

effectively digest cell-wall ploysaccharides.

In-Fruit Seed Germination in Grapefruit

In-fruit seed germination often occurs during the late season (Purvis and Albrigo, 1984) and may become severe during storage or transit (Albrigo et al., 1980, 1981). Little is known about potential factors associated with seed germination within fruit.

In general, it is accepted that citrus seeds do not require a dormancy period before germination can occur (Schneider, 1968). Water-soaks, low temperature, or gibberallic acid treatment accelerated the germination rates or shortened the time required for germination of orange seeds (Burns and Coggins, Jr., 1969; Burger and Hackett, 1982). The seed coat was also thought to be a germination barrier in citrus because germination following removal of the seed coat was completed within 5-7 days (Monselise, 1962). These data have been interpreted as evidence for the presence of leachable inhibitors in the seed coat. In grapefruit, seeds can germinate when isolated from the fruit after August in Texas, at which time fruit are still immature (Pucik, 1974). Although abscisic acid (ABA) is associated with seed rest in many seed types, exogenous ABA has no effect on the germination of seeds isolated from the fruit after February (Purvis and Albrigo, 1984). This indicates that seeds in overmature fruit had already passed

the developmental stage affected by ABA. Albrigo (unpublished data) found that scoring the seed coat accelerated the germination of grapefruit seeds as much as cosplata seed coat removal. Germination was retarded only when seed coats were intact. These data are inconsistent with the idea that inhibitors are present in the seed coat. The delayed germination with intact seed coats appeared to be associated with the physical resistance of tough testa or impermeability of the inner seed coat to water.

Moisture content of seed in general plays an important role in seed maturation and germination (Adams and Rinne, 1980; Karmoda et al., 1986). The final stage of development in most seeds is dehydration, which results in termination of synthesis of reserve materials; however, water loss below a certain critical point results in a severe loss of viability in citrus seeds (Nauer, 1981). Severe water loss effecting viability cannot be expected in the fruit. In trifoliata orange seed, water content decreased when fruit became mature, and then increased slightly in overripe fruit even though the osmotic potential of juice decreased from $-850\ J.kg^{-1}$ to $-1300\ J.kg^{-1}$ (Mobayen, 1980). This may indicate that the seed can absorb water under this osmotic environment or that the water may have originated from the vascular system. Purvis and Albrigo (1984) found that the use of grapefruit juice as the germination medium did not retard germination of extracted grapefruit seed. Since water movement in juice vesicles is limited, leaked juice

resulting from juice vesicle disorders may be a water source for in-fruit germination of grapefruit seeds if the water potential of seed at maturity is low enough to cause a net movement of water into the seeds. As mentioned earlier, the reduction of TSS and acidity is characteristic of disordered juice-vesicles. Thus, it can be assumed that low TSS and acidity in vesicle disordered fruit can contribute to the generation of a satisfactory water potential gradient from juice to seed.

MATERIALS AND METHODS

Experiment 1: Occurrence of Section Drying (SD) and In-Fruit Germination in Late-Season Grapefruit

Plant Materials

Grapefruit of two cultivars (*Citrus paradisi* Macf. cv. Marsh seedless and Ruby Red) were harvested from 4 groves for 3 successive years (1986-1988) for each cultivar. All groves were located in the Indian River area of Florida.

Fruit Harvest and Storage Treatment

Sixty fruit were harvested monthly from each of the same 3 trees (March through May, 1986, 1987, and 1988). Half of the fruit were examined immediately for vesicle disorders. The remaining fruit were examined after 40 days storage at 21°C. Before storage, fruit were washed, treated with 600 ppm benomyl [methyl 1-(butyl-carbamoyl)-2-benzimidazole-carbamate] as a non-recovery spray, dried and waxed with solvent wax.

Determination of Vesicle Disorders

Section drying (SD) was examined from 3 cut surfaces perpendicular to the core axis [stem-end (approximately 1/3 of the distance from the stem, center (approximately equatorial), and styler-end (2/3 of the distance from the

stem-end)]. Severity of the disorder was recorded from a range of 0 (no appearance of SD) to 3 (more than 2/3 of the cut surfaces). Severity was numerically expressed as the sum of graded severity from each cut surface per fruit portion divided by maximum possible severity per cut surface. Total severity was expressed as the sum of severity of the 3 cut surfaces. Type of vesicle disorder [granulation (G) or vesicle collapse(VC)] was recorded for each cut surface in 1987 and 1988. In 1988, no distinction was made regarding disorder type.

Determination of In-fruit Germination

After examining the fruit for vesicle disorders, seeds were extracted and germinating seeds were counted. Seeds with split seed coats were considered to be germinating.

Water and Abscisic Acid (ABA) Injection into Fruit

'Marsh Seedless' grapefruit were harvested on May, 1987. After storage treatments as described above, 50 fruit without any blemishes were selected. Before water or ABA injection, the fruit surface was sterilized with 70% ethanol. Approximately 2.5 to 3.5 ml of sterile water or ABA (4×10^{-5} M) were injected into each fruit (core axis) with a sterilized syringe. Fruit not injected were used as controls. Fifty fruit were examined immediately (no treatment). Fruit with other treatment were stored at 21°C for 30 days and germinating seeds were counted from the sound fruit as described earlier.

Film-Wrapping of Fruit

Low-density-polyethylene film (shrink-film), with heat sealed seams, was applied with a wrapping machine and hot-tunnel shrunk at 200°C. Control fruit were washed and waxed as described earlier. Fruit were stored at 15.5°C, 21°C, and 29.4°C. Vesicle disorders were examined after 62 days storage. Decayed fruit during storage were discarded.

Experiment 2: Comparative Study of Cell Walls in Disordered Juice-Vesicles in Grapefruit

Plant Materials

Normal and disordered juice-vesicles were isolated from disordered grapefruit (Citrus paradisi Macf. cv. Marsh seedless) stored at 21°C for 40 days. Juice-vesicles which showed no visible symptoms of granulation or vesicle collapse were regarded as normal vesicles. Isolated vesicles were stored in polyethylene bags at -20°C.

Determination of Fresh Weight, Dry Weight, and Moisture Contents

Fifty juice-vesicles were isolated from stored fruit, and weighed and divided by the number of vesicles to determine the average weight of one vesicle. After measurement of fresh weight, juice-vesicles were dried in an oven at 67-70°C. Moisture contents were calculated as a percentage of dry matter over fresh weight. This experiment was done with 3 replications (50 vesicles per replication).

Determination of Acids in Juice

Acid analysis was conducted from the juice squeezed by hand. Five ml of hand-squeezed juice were diluted to 50 ml with deionized water and titrated by adding 0.05 M NaOH. Titration end point (pH 7.0) was determined using a pH meter. Acidity was expressed as percentage juice as citric acid.

Determination of Soluble Sugars

Fifteen grams of vesicle tissue were homogenized in 80% ethanol (30 ml) for 3 min using a Sorval Omnimixer at full speed. The homogenate was placed in a bottle in a water bath at 60°C for 1 hr. After collecting the ethanol fraction, soluble sugars were further extracted with fresh alcohol (50 ml) for another 1 hr. The combined ethanol soluble fractions were filtered through glass fiber filter paper (GF/C filter paper, Whatman) and total volumn was adjusted. Sugar was analyzed from the filtrates by the phenol-sulfuric procedure (Dubois et al., 1956). D-glucose was used as the standard.

Preparation of Ethanol-Insoluble-Solids (EIS)

Ethanol-insoluble-solids (EIS) were prepared as described by Huber (1984), with slight modification. Forty five grams of vesicle tissue were homogenized in 190 ml 95% ethanol for 3 min using a Sorval Omnimixer set at full speed. The homogenate was refluxed for 20 min in a boiling water bath and then stored overnight at -20°C. The suspension was filtered through Miracloth (Biochemical Corp.

La Jolla, CA) and washed with 250 ml of 80% ethanol (v/v), followed by 250 ml of 80% acetone (v/v). The recovered EIS were suspended in 100 ml of 100% acetone, and filtered through glass fiber filter (G/F C filter paper, Whatman) under slight aspiration on a millipore funnel. An additional 400 ml of 100% acetone was added and EIS were stirred under aspiration until filtration was complete. Ethanol-insoluble-solids were transferred to petri dishes and excess acetone evaporated at room temperature. Ethanol-insoluble-solids were dried overnight at 35°C and stored in a desiccator at room temperature.

Isolation and Determination of Polyuronides and Hemicelluloses

Soluble polyuronides and hemicelluloses were prepared basically as described by Huber (1984) and Huber and Lee (1988). Water-soluble polyuronides were isolated by suspending EIS (100 mg) in 35 ml of deionized water. The suspension was placed on the shaker for 8 hours at room temperature. After 6 hours, the suspension was filtered through Miracloth and washed with 10 ml of deionized water. The combined filtrates were further filtered through glass fiber filter paper (GF/C, Whatman) under aspiration. The filtrates were stored at -20°C until used. Uronic acid was determined as described by Blumenkrentz and Asboe-Hansen (1973). D-galacturonic acid was used as the standard.

After extraction of water-soluble polyuronides, the recovered powder was placed in 35 ml of Na-acetate (40 mM, pH 5.0) containing 20 mM Na_2EDTA and shaken for 6 hours.

The suspension was again filtered through Miracloth and washed with 10 ml of extraction buffer. The combined filtrates were further filtered through glass fiber filter paper under aspiration and stored at -20°C. Acid sugars in EDTA soluble polyuronides were measured by the method of Blumenkrantz and Asboe-Hansen (1973).

Prior to hemicellulose extraction, polyuronides were removed from the EIS by placing 100 mg EIS in 150 ml Na-phosphate (100 mM, pH 6.5) containing 20 mM Na_2EDTA. The suspension was then placed in a boiling water bath for 30 min. After cooling to room temperature, the suspension was filtered through Miracloth, and then washed with 150 ml of extraction buffer, followed by 500 ml of deionized water. Hemicelluloses were extracted from the recovered powder by suspending the powder in 15 ml of 4 N NaOH containing 6 mg $NaBH_4 \cdot ml^{-1}$. After two successive 4 hour extractions, the alkali-soluble fractions were filtered through Miracloth. The combined filtrates were neutralized with concentrated acetic acid (1°C) and dialyzed against running tap water (12 hrs, at room temperature), 4 L of 10% methanol (12 hrs, 4°C), and finally with 4 L of deionized water (2 x 12 hours, 4°C). After dialysis, the samples were filtered through glass fiber filter paper (GF/C, Whatman). The filtrates were stored at -20°C. Hexoses were measured by the phenol-sulfuric procedure (Dubois et al., 1956). D-galactose was used as the standard.

Total polyuronides in EIS were measured by the method described by Ahmed and Labavitch (1977). Five to six mg EIS were placed in 30 ml beakers in an ice bath for 5 min. Afterwards, 2.5 ml of chilled, concentrated sulfuric acid were pipetted slowly onto the EIS. After 5 min, the samples were stirred for 5 min and 0.7 ml chilled deionized water was added. After another 5 min, an additional 0.7 ml of chilled water was added. After a final 5 min period, 35 ml of deionized water were added. Aliquots (0.5 ml) were analyzed for uronic acid sugars (Blumenkrantz and Asboe-Hansen, 1973).

Determination of cellulose

Cellulose content in EIS was determined as described by Updegraff (1969). Twenty mg of EIS were placed in 5 ml of acetic acid : nitric acid : H_2O (4/1/2, v/v/v) and placed in a boiling water bath for 30 min. The suspension was centrifuged in a clinical centrifuge for 10 min at full speed (490g). The supernatant was discarded. The pellet was washed with 10 ml of deionized water (2 X) and centrifuged. The pellet after the final centrifugation was hydrolyzed in 67% sulfuric acid for 1 hr. Total hexoses (D-glucose equivalent) were measured by the anthrone-sulfuric acid procedure (Hodge and Hofreiter, 1962).

Gel Filtration Chromatography of Soluble Polyuronides and Hemicelluloses

Gel-filtration of polyuronides was conducted on a bed (60 cm high, 1.5 cm wide) of Ultrogel AcA 22 (Bio-Rad Lab., Richmond, CA) packed in Na-acetate buffer (25 mM, pH 5.0)

containing 3 mM Na_2EDTA and 100 mM NaCl. Approximately 2 mg polyuronides in 2 ml of extraction medium (water or buffer) were applied to the column and gravity eluted at a flow rate of 17.5 $ml \cdot cm^{-2} \cdot hr^{-1}$. Half-ml aliquots of the 2 ml fractions were analyzed for acid sugars by the procedure of Blumenkrantz and Asboe-Hansen (1973).

Hemicellulose (approximately 4 mg) in 2 ml of deionized water was applied to a column (60 cm high, 1.5 cm wide) packed with Ultrogel AcA 34 (LKB) in Na-acetate buffer (25 mM, pH 5.0) containing 3 mM Na_2EDTA and 100 mM NaCl. Hemicellulose was gravity eluted at a flow rate of 63 $ml \cdot cm^{-2} \cdot hr^{-1}$. Fractions of 2 ml were collected and assayed for hexose by the phenol-sulfuric acid procedure (Dubois et al., 1956).

Neutral Sugar Analysis of Polyuronides and Hemicelluloses

Prior to neutral sugar analysis of polyuronides, samples were first subjected to ion-exchange chromatography to remove nonstructural neutral sugars. Ion-exchange chromatography was carried out on a bed (16 cm high, 1.5 cm wide) of DEAE-Sephadex (Sigma Chemical Co., St. Louis, MO) which was equilibrated and packed in Na-phosphate buffer (15 mM, pH 6.8) containing 20 mM NaCl and 5 mM Na_2EDTA. Following dialysis against the column buffer, 4 mg of polyuronides were applied to the column. After 100 ml of elution buffer were collected, polyuronides were eluted by a salt gradient (0.8 M NaCl in the elution buffer).

Polyuronides recovered following ion-exchange were dialyzed against deionized water for 24 hours at 4°C. One mg of polyuronides was air-dried in Reacti-Viale (Pierce Chemical Co., Rockford, IL), and hydrolyzed and acetylated as described by Loecher and Nevins (1972). The derivatized sugars were examined by gas chromatography on a glass column packed with 3% SP 2340 (SupelCo, Inc., Bellefonte, PA). Oven temperature was 225°C and helium was used as the carrier gas. myo-Inositol was used as the internal standard (Loscher and Nevins, 1972).

Neutral sugars in hemicelluloses were analyzed after passing through the DEAE-sephadex column described in the above. Hemicelluloses eluted under the initial buffer strength were collected and followed the procedures described in the above.

Polygalacturonase Extraction and Assay

Fifteen grams of partially thawed vesicle tissue were homogenized in phosphate buffer (200 mM, pH 6.0) containing 2.4 M NaCl for 3 min at full speed using a Sorvel Omnimixer. The homogenate was incubated for 1 hr and then centrifuged for 20 min at 32,000g. Proteins were precipitated from the supernatant by adding solid ammonium sulfate to 75% saturation. The precipitated protein was centrifuged for 20 min at 32,000g. The supernatant was discarded. The pellet was dissolved in 2.5 ml of Na-acetate buffer (50 mM, pH 5.0) and desalted by passing through a PD-10 column packed with Sephadex G-25 M (Pharmacia, Sweden). Protein content was

determined as described by Bradford (1976), using bovine serum albumin as the standard.

Determination of Lignin Components in Ethanol-Insoluble-Solids (EIS)

Twenty mg of EIS were hydrated with deionized water under vacuum for 1 hr. The hydrated EIS were spread on a glass microscope slide and lignin components stained by adding 2-3 drops of 1% phloroglucinol in 18% HCL. After 10 min, stained spots were examined under a light microscope. Stained spots were recorded from 10 microscopic fields with 3 replications.

Scanning Electron Microscopy

Disordered and normal juice-vesicles were isolated from fruit stored at 21°C for 40 days or more. Vesicles were prefixed in 2% glutaraldehyde in phosphate (100 mM, pH 7.2) for 2 hours and then postfixed in 2% osmium tetroxide in the same buffer. Specimens were washed with deionized water for 2 hours, dehydrated using an ethanol series and critical point dryer (Ladd Industry). After critical point dried in CO_2, vesicles were cut with a razor blade, mounted with the cut surface up, and sputter-coated with gold. Specimens were examined with a Hitachi scanning electron microscope (S-530) at 20 kV of accelerating voltage .

RESULTS AND DISCUSSION

I. Occurrence of Section Drying (SD) and In-Fruit Germination in Late-Season Grapefruit

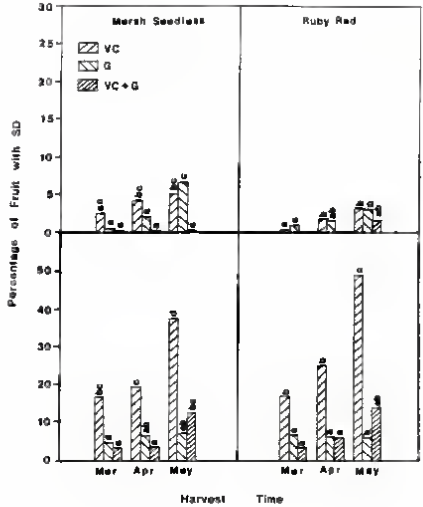
Introduction

Vesicles in SD grapefruit showed symptoms similar to core dryness or dry juice sac in oranges as described by Bartholomew et al. (1941) and Noort (1969). Thus, juice-vesicles affected by vesicle collapse showed signs of shrinkage at the initial stages and they collapsed completely at the final stages. No hardening symptoms were observed at any developmental stages. At advanced stages of VC, the affected juice vesicles become very thin due to loss of tissue fluids. Granulation was also found in the SD fruit; however, G was usually restricted to the vesicles at the stem- or stylar-end. Grapefruit were not usually affected by VC in the early stage of fruit maturation whereas G was observed in the early season in some vesicles just beneath the peel tissue of the stem- or stylar-end.

Types of Section Drying in Grapefruit

It appeared that the primary vesicle disorder in grapefruit was VC (Fig. 1). In freshly harvested fruit, there was a progressive increase in both G and VC as the season progressed. The variation between harvest dates was

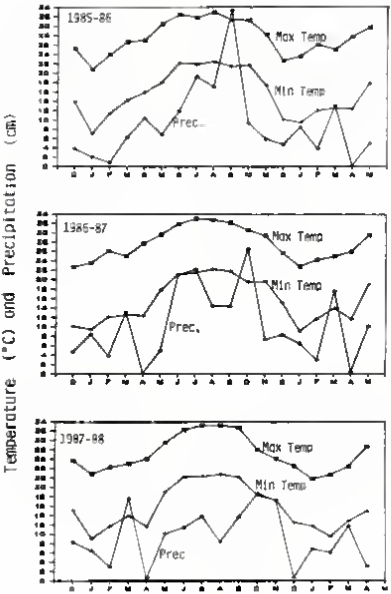
Fig. 1. Occurrence of vesicle collapse (VC), granulation (G), and both in 'Marsh Seedless' and 'Ruby Red' grapefruit. Results are from the pooled data of 1987 and 1988. A and C: at harvest, B and D: after storage for 40 days at 21°C. Different letters within a cultivar (A or C) or storage time (B or D) indicate significant difference by Duncan's multiple range test (p=0.05).


Marsh Seedless
Ruby Red
VC
G
VC+G
Percentage of Fruit with SD
Mar
Apr
May
Harvest Time

significant but the level of either VC or G was less than 10% at all harvest dates (Fig. 1A and C). VC was slightly higher than G in 'Marsh Seedless' at April harvest. The disorder was slightly higher in 'Marsh Seedless' than 'Ruby Red' but the differences between vesicle disorder types in general was not significant. After storage, the incidence of VC became much greater than G in both cultivars (Fig. 1B and D). The May-harvested fruit in both cultivars developed the highest amount of disordered fruit (Fig. 1B and D). Fruit affected by G or both disorders also increased during storage but not to the extent of fruit affected by VC alone. Bartholomew et al. (1941) also reported that grapefruit were less affected by G compared to oranges.

As mentioned earlier, dry juice-sac was proposed to be associated with excess water loss caused by drought during fruit development (Sinclair, 1984). In this study, climatic data did not show consistent trend. The monthly precipitation averages during the late maturation period (December-February) in 1985-86 and 1986-87 were 56 mm and 59 mm, respectively, whereas it was 45 mm in 1987-88 (Fig. 2). Although the precipitation in 1985-86 and in 1986-87 showed little difference, SD was much decreased in 1986-87. Also, the cumulative precipitation of March and April of each year, which might have influenced the late-harvest fruit, were 133 mm, 182 mm, and 150 mm in 1986, 87, and 88, respectively. These data seem to agree with the previous proposal since the lowest SD year (1987) received highest

Fig. 2. Monthly average minimum and maximum temperature and average precipitation during the growing season (December, 1986- May, 1988) of grapefruit. Data are the average from 4 locations near experimental area.


1985-86
Max Temp
Min Temp
Prec.
1986-87
Max Temp
Min Temp
Prec.
1987-88
Max Temp
Min Temp
Prec
Temperature (°C) and Precipitation (cm)
Month

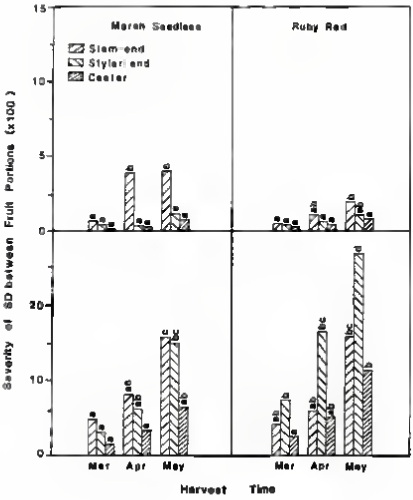
precipitation. To make a concrete conclusion, it requires to perform a better designed experiment.

The above observations indicate that VC is the primary vesicle disorder resulting in SD of grapefruit and grapefruit may severely suffer from SD in late harvest and/or storage and transit.

Localization of SD Incidence within Fruit

The incidence of SD among fruit portions somewhat differs in the two cultivars. In 'Marsh Seedless' at harvest, the stem-end was slightly more affected than other fruit portions except in the March-harvested fruit (Fig. 3A). This tendency of SD incidence continued even after storage (Fig. 3B). However, significant differences were only observed in the May-harvested fruit. Both the stem- and stylar-end in stored fruit were nearly evenly affected by SD and was significantly more affected than the central fruit portions (Fig. 3B). In 'Ruby Red,' no difference was found at all harvest dates before storage except the May-harvest fruit at which SD was slight higher at the stem-end (Fig. 3 C). After storage, the stylar-end tended to be more affected than other fruit portions, especially in May-harvest fruit (Fig. 3D). Unlike core-dryness in navel oranges, the central portion of the fruit was least affected in both cultivars at harvest and even after storage, but SD in central fruit portions was never significantly lower than both the stem- and stylar-end SD in grapefruit differed from dry juice sac in oranges. Dry juice-sac usually occurs

Fig. 3. Severity of section drying (SD) in different fruit portions of 'Marsh Seedless' and 'Ruby Red' grapefruit. Results are from the pooled data of 1986-1988. A and C: at harvest, B and D: after storage for 40 days at 21°C. Different letters within a cultivar at harvest (A or C) or after storage (B or D) indicate significant difference by Duncan's multiple range test (p=0.05).


Marsh Seedless
Ruby Red
Stem-end
Stylar-end
Center
Severity of SD between Fruit Portions (x100)
Harvest Time
Mar
Apr
May

at the stylar end of small orange fruit (Bartholomew et al., 1941). The results indicate that SD (primarily VC) may occur at any portion of the fruit but both the stem- and stylar-end may be more affected in senescant fruit.

Since vesicle disorders occurs in the late season, it has been proposed that G and other juice-vesicle disorders are associated with fruit senescence (El-Zeftawi, 1976). It is not known whether the difference in the incidence of SD between fruit portions results from differences in tissue metabolism or in the degree of tissue senescence between fruit portions. Sugar and acid gradients between fruit portions may be an indirect indication of differences in tissue metabolism between fruit portions. Sugars and acids contents are low in the stem-end of citrus fruit (Ting, 1969). Matsumoto (1964) examined the respiration rate (on the basis of CO_2 production and O_2 absorption) among fruit portions (stem-end, stylar-end, and central portion) in mature oranges; respiration of fruit tissue was highest at the stem-end, intermediate at the stylar-end, and lowest at the central fruit portion. This respiration difference may result in the low levels of sugars and acids at the stem-end. Cold storage-temperature was effective on the reduction of G in oranges (El-Zeftawi, 1976) and SD in grapefruit (Albrigo et al., 1980, 1981). This observation can be interpreted that the decrease in vesicle disorders may be the result of delaying of physiological events in fruit by low temperature. Thus, it is reasonable to assume

that high air-temperatures at the mature stages of grapefruit may be associated with the occurrence of SD. However, climatic data did not show large differences in the late seasons (December-April, each year) (Fig. 2). In 1986, grapefruit produced in Florida contained less acids and total soluble solids (TSS) (Fig. 4) and developed relatively high amount of SD (Fig. 5), whereas in 1989, grapefruit contained relatively high acids and TSS but developed a similar amount of SD as in 1986 (Fig.7).

Variation of SD between Crop Years and Groves

It appeared that significant differences existed in the amount of SD between groves and years (1986, 1987, and 1988) (Fig. 5, 6, 7) after storage, although the incidence of SD did not differ significantly at harvest (Duncan's multiple range test, p= 0.05). Results of variance analysis are summarized in Table. 1. In 1986, the section drying range was 0 to 26% at harvest, and in 1987 and 1988, the ranges were 0 to 13% and 0 to 25% at harvest, respectively. After storage, the variation was greater; 13-87% in 1986 and 1988, and 3-68% in 1987. The amount of SD fruit was low in 1987 compared to the other 2 years. Interestingly, fruit from grove 8 consistently exhibited the highest amount of SD although the difference was not significant at harvest. Grove 2 exhibited a relatively small amount of SD during the experiment. The other 6 groves showed intermediate or inconsistent amounts of SD between years. A genetic factor was proposed to be associated with the incidence of G in

Fig. 4. Total percent soluble solids (°Brix) and acids of grapefruit produced in Florida (1985-86, 1986-87, and 1987-88 seasons). Data were obtained from Annual Report, Fruit and Vegetable Inspection, USDA, Winter Haven, Florida.

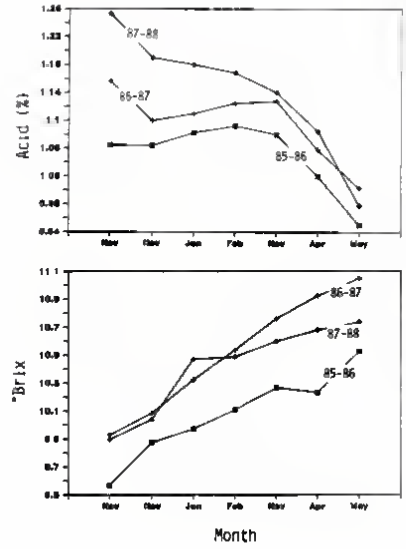

Acid (%)
87-88
86-87
85-86
Nov
Dec
Jan
Feb
Mar
Apr
May
°Brix
86-87
87-88
85-86
Month

Fig. 5. Variation in percentage of fruit with section drying (SD) in 'Marsh Seedless' and 'Ruby Red' grapefruit harvested on 3 dates during the late season (March, April, and May) in 1986. A; March, B; April, C; May. Different letters within a cultivar and a harvest time (March, April, or May) indicate significant differences by Duncan's multiple range test ($p=0.05$).

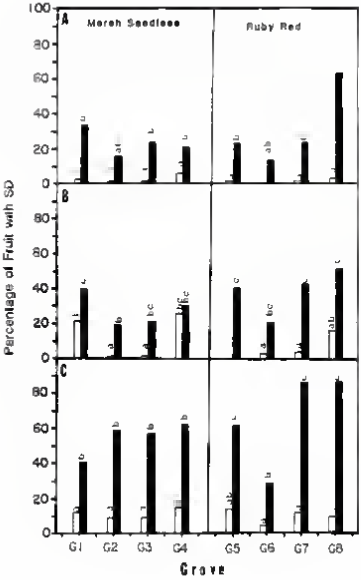

Marsh Seedless
Ruby Red
A
B
C
Percentage of Fruit with SD
100
80
60
40
20
0
G1
G2
G3
G4
G5
G6
G7
G8
Grove

Fig. 6. Variation in percentage of fruit with section drying (SD) in 'Marsh Seedless' and 'Ruby Red' grapefruit harvested on 3 dates during the late season (March, April, and May) in 1987. A, March, B; April, C, May. Different letters within a cultivar and a harvest time (March, April, or May) indicate significant differences by Duncan's multiple range test ($p=0.05$).

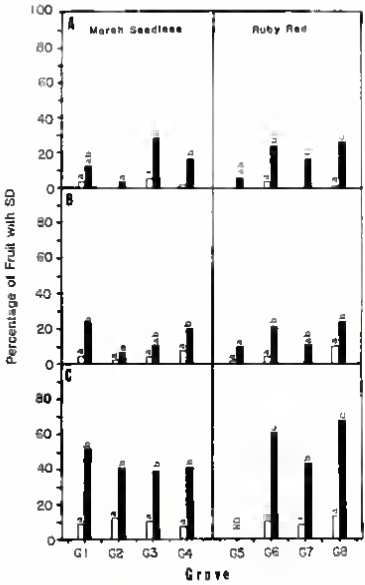

A
Marsh Seedless
Ruby Red
B
C
Percentage of Fruit with SD
100
80
60
40
20
0
ND
G1
G2
G3
G4
G5
G6
G7
G8
Grove

Fig. 7. Variation in percentage of fruit with section drying (SD) in 'Marsh Seedless' and 'Ruby Red' grapefruit harvested on 3 dates during the late season (March, April, and May) in 1988. A; March, B; April, C; May. Different letters within a cultivar and a harvest time (March, April, or May) indicate significant differences by Duncan's multiple range test (p=0.05).

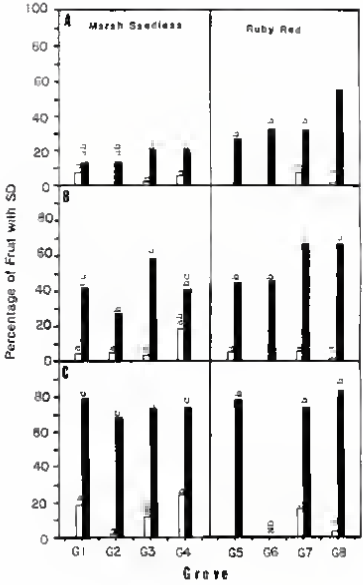

A
Marsh Seedless
Ruby Red
B
C
Percentage of Fruit with SD
100
80
60
40
20
0
ND
G1
G2
G3
G4
G5
G6
G7
G8
Grove

Table. 1. Analysis of variance for several factors affecting section drying of grapefruit harvested from Indian river groves, Florida.

Source	df	F (Observed)	F (Required, 1%)
Year (Y)	2	20.18	8.51
Grove (G)	7	5.09	4.28
Harvest Time (H)	2	108.08	5.43
Storage (S)	1	325.25	7.19
Interaction			
G X H	14	1.02	2.70
G X S	7	3.33	3.04
Y X H	4	24.30	5.08
G X H X S	14	0.46	2.48

Note: Analysis was carried out from the pooled data of 3 years (1986-1988) and 2 grapefruit cultivars ('Marsh Seedless' and 'Ruby Red').

some oranges (Bartholomew et al., 1941: Awasthi and Nauriyal, 1972) because some trees within a cultivar or some cultivars consistently produced more granulated fruit. Whether the high amount of SD in grove 8 is related to some inherent genetic characteristics is not known.

The variations in the incidence of G between years and locations have been regarded as the result of environmental effects (Bartholomew et al., 1941, Noort, 1969). Because fruit were harvested from the same trees, the great variation between crop years in this study seems to indicate that environmental conditions are associated with SD. Noort (1969) reported that G and other vesicle disorders increased following a relatively warm winter. However, results of this experiment were not consistent with this idea; the trees were exposed to a relatively cold winter in 1966 (December, 1965-February, 1966) but developed the highest SD of the 3 years even though there was no indication that fruit experienced freeze injury in 1966 (Fig. 5). On the other hand, in 1967, the trees experienced a relatively warm winter (December, 1966-February, 1967) but produced a relatively low amount of SD fruit (Fig. 6).

Film wrapping of individual fruit has advantages in several citrus fruit types primarily because of alleviation of water stress (Ben-Yehoshua et al., 1983; Purvis, 1983). Wrapping in low-density polyethylene film did not effect the development of SD in grapefruit (Table. 2). Only low storage temperatures appeared to decrease SD in this test

Table 2. Effect of storage temperature and plastic film wrapping on section drying and in-fruit germination of 'Marsh Seedless' grapefruit.

Storage Temp. (°C)	Wrapping	Fruit No.	SD (% of Fruit)	Germ. (% of Fruit)
15.5	-	53	56.7	15.9
	+	55	28.8	12.7
21	-	45	51.1	22.2
	+	56	57.2	23.2
29.4	-	51	72.5	37.3
	+	50	78.0	60.0

Note: Fruit were harvested on May, 1987 and SD was examined after 62 days storage.

indicating that water loss is not a primary factor associated with SD in grapefruit. Water loss has been proposed to be a prima factor associated with dry juice-sac in oranges (Sinclair, 1984). Albrigo et al. (1981) also reported that film-wrapping did not prevent SD incidence of grapefruit during storage or transit.

Incidence of In-fruit Germination

In-fruit germination often occurs in fruit harvested late in the season (Albrigo et al., 1980; Purvis and Albrigo, 1984). Seedless grapefruit cultivars commercially grown in Florida still contain 3-4 viable seeds (Albrigo and Carter, 1978) which often germinate during storage or transit. In-fruit germination results in off-flavors and odors (Albrigo and Carter, 1978), which is not desirable for fresh market or processing. Thus, fruit containing sprouting seeds cannot be used for juice extraction (Florida Department of Citrus, 1975).

The average percentage of fruit affected by in-fruit germination in both cultivars for 3 years is summarized in Tables. 3 and 4. There was an increase in seed germination within fruit as the season progressed. 'Ruby Red' showed a slightly higher incidence of in-fruit germination but the differences were not significant.

Since juice components in citrus are located in the large vacuolated cells which are enclosed by a tough, skin-like, cuticle-coated epidermis (Schneider, 1968; Fahn, 1974), water movement is limited once stored in the juice

Table. 3. In-fruit germination of 'Marsh Seedless' grapefruit harvested on 3 dates during the late seasons in 1986, 1987, and 1988.

Harvest Time	Treat.[x]	Year			AVG
		86	87	88	
		% of Fruit			
Mar	H	0.28a[y]	0.00a	0.00a	0.09
	S	8.42a	1.69a	0.98a	3.70
Apr	H	2.35a	0.28a	0.60a	1.08
	S	34.83b	5.57a	4.64a	15.01
May	H	8.42a	3.19a	2.03a	4.55
	S	31.78b	27.18b	19.40b	26.12
AVG	H	3.68	1.16	0.88	
	S	25.01	11.48	8.43	

[x] H and S represent harvest and stored, respectively.
[y] Different letters within a column indicate significant difference by Duncan's multiple range test (p=0.05). Numbers are the average percentage of fruit containing sprouting seeds with 4 replications (1 replication per grove).

Table. 4. In-fruit germination of 'Ruby Red' grapefruit harvested on 3 late seeeone in 1986, 1987, and 1988

Harvest Time	Treat.[z]	Year			AVG
		86	87	88	
		%			
Mar	H	0.00a[y]	0.38a	0.10e	0.16
	S	30.80b	18.28e	13.21ab	20.76
Apr	H	7.82a	0.56e	0.61e	3.00
	S	42.61b	21.29eb	12.00ab	25.30
May	H	9.38a	4.24a	2.09a	5.24
	S	44.45b	44.32b	17.90b	35.56
AVG	H	5.73	1.73	0.93	
	S	39.29	27.96	14.37	

[z] H and S repreeent et harvest end etored fruit, reepectively.

[y] Different lattara within column indicate eignificent difference by Duncan's multiple range test (p=0.05). Numbers are the avearge percentage of fruit contaning eprouting eeede with 4 replicatione (1 replication per grove).

cells (Kaufmann, 1970). However, it has been suggested that in-fruit germination is caused by the leakage of tissue fluids (Purvis and Albrigo, 1984). To examine free water effects on seed germination in the fruit, sterilized water or abscisic acid (ABA) (4×10^{-5}) was injected into fruit. Water injection increased germination (Table. 5), whereas germination was not affected by exogenous ABA. These results indicated that seeds in overmature fruit could germinate when a water source is available. Also, seeds in mature fruit had already passed a certain developmental stage at which they are sensitive to ABA (Purvis and Albrigo, 1984).

Correlations between SD and in-fruit germination were examined. High correlations (T-test, p=0.01) were observed each year between the total severity of SD and in-fruit germination in both cultivars although there was a variation in the amount of fruit containing sprouting seeds between years (Tables. 3 and 4). Results of simple regression (r) were 0.75, 0.79, and 0.70 in 1985, 87, and 88, respectively. Results of 1986 and 1988 were shown in Fig. 8 since data obtained in 1987 was nearly identical to 1986. Even though strong correlations existed, r^2 values accounted for only 50% of total variance in in-fruit germination suggesting that other factors were involved.

Total-soluble-solids (TSS) and acid content of juice seem to be related to in-fruit germination. The average in-fruit germination of fruit from 3 harvests was highest in

Fig. 8. Relationship between severity of section drying (SD) and precocious germination in grapefruit. Results in 1986 and 1988 are from the pooled data of each year and 2 cultivars. Total severity is the sum of severity from 3 cut surfaces (X100).

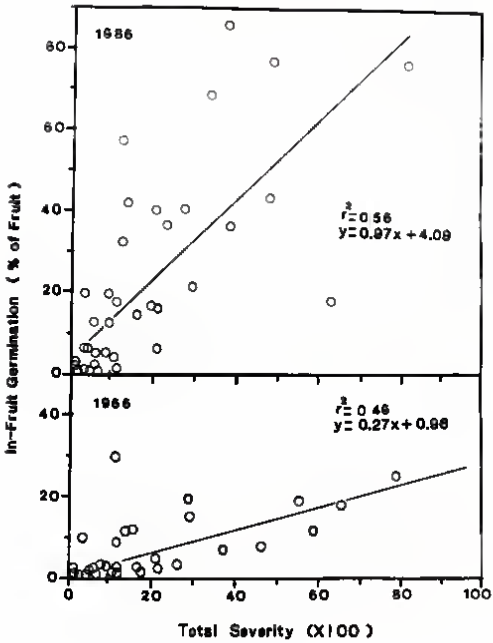

1986
r^2= 0.56
y= 0.97x + 4.09
1966
r^2= 0.46
y= 0.27x + 0.96
In-Fruit Germination (% of Fruit)
Total Severity (X100)
0
20
40
60
80
100

Table. 5. Effect of water and ABA injection into fruit on the in-fruit germination of 'Marsh Seedless' grapefruit

Treatment[z]	Fruit Number	Total Seed Number	Germinating Seeds
Before Storage	50	218	0 (0.0)[y]
Stored	49	223	3 (1.4)
Water	29	132	22 (18.7)
ABA	28	133	25 (18.8)

Note: Grapefruit were harvested on May, 1987.

[z] Before storage; germination at harvest, stored; germination after storage without treatment, water and ABA injection; 2.5-3.5 ml of sterilized water or ABA (5×10^{-3} M) was injected. Germination was examined after 30 days storage at 21°C.

[y] Numbers in parentheses represent percentage of germination.

1986 (Table. 3,4), when TSS and acid content of juice were lowest for the 3 years (Fig. 4). On the other hand, in 1988, in-fruit germination was lowest whereas TSS and acid contents were high (Fig 4). If the leaked juice is the source of water for in-fruit germination, it is reasonable to expect that low TSS and acid contents may result in a more favorable water potential gradient between juice and seeds, and it would be easier for seeds to take up water from the leaked juice. The fact that in-fruit germination occurs only in the late season supports this hypothesis because seeds in overmature fruit might pass the dehydration stage of seed development (Mobayen, 1980) and thus water potential of the seed would be low. It is also possible that low in-fruit germination in 1988 may have resulted from low water potential gradient between seeds and juice. When water potential gradients are small, water absorption by seeds may occur slowly. Mobayen and Milthrope (1978) demonstrated that in the trifoliate oranges, seed germination was delayed under high salinity conditions. However, the water potential of the medium (-750 Jk^{1}) did not effect the final germination percentage.

The strong acidity of citrus juice was proposed to be a potential inhibitor for citrus seed germination (Monselise, 1962). Whether the high acidity of juice in 1988 fruit contributed to the reduction of germination is not known. Since TSS and acidity of fruit are dependent on the rainfall and/or temperature in the growing season,

especially during maturation, these environmental factors should be considered. During the period of December through March, the total precipitation in 1985-86 and 1986-87 was 300 mm and 355 mm, respectively, whereas it was 155 mm in 1987-88. This lower precipitation in 1987-1988 may have resulted in the higher TSS and acid content of fruit.

Purvis and Albrigo (1984) suggested a possible relationship between low temperature (below freezing point) during winter and an early germination within fruit probably due to the degradation of juice-cells by freeze damage. In this study, fruit did not experience any freeze damage. Since cold treatment of isolated seeds stimulates the germination of 'Valencia' orange seeds (Burger and Hackett, 1982), the number of days below 4°C was examined. The number of days below 4°C (minimum temperature) was 12.3 days in 1985-86 during December through February while it was 7.8 days and 8.0 days in 1986-87 and 1987-88, respectively. It does appear that the year of the highest in-fruit germination was the year with coldest winter. Whether or not low temperatures during winter stimulated in-fruit germination is not known.

Individual wrapping did not affect precocious germination although low storage temperature reduced precocious germination (Table. 2). This observation agrees with the previous observations (Albrigo et al., 1981; Purvis and Albrigo, 1984). Low temperature seems to suppress the germination by delaying the general metabolism of the seeds

during storage of fruit. However, neither respiration nor other metabolic events in the seeds have been examined.

II. Comparative Study of Cell Walls in Disordered Juice-Vesicles in Grapefruit

Introduction

Structural modifications of fruit cell walls is one of the characteristic physiological changes occurring during ripening. Changes affecting cell walls include an increased solubility of specific cell-wall polysaccharides (Huber, 1983a; Brady, 1987), and a decrease in neutral sugar content (Gross and Sams, 1984). These events may also be associated with the loss of tissue integrity occurring in ripe fruit affected by physiological disorders (Elkashif and Huber, 1988).

In citrus, juice-vesicle disorders have been known since the 1900's. In 1941, Bartholomew et al. thoroughly documented granulation (G) in 'Valencia' oranges. Unlike normal vesicles, granulated vesicles produce more cell-wall polysaccharides, increase in vesicle size and texture. Among the cell-wall components, pectins in particular have been proposed to be a potential factor associated with the hardening of granulated vesicles (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961). The occurrence of starch in granulated fruit (Chakrawar and Singh, 1977b) indicates that an alteration of carbohydrate metabolism may be of

importance for the development of G.

In addition to G, juice-vesicle dehydration occurs in oranges and manderin-type citrus (Bartholomew et al., 1941; Matsumoto, 1964; Sinclair, 1984). This disorder once regarded as an different expression of granulation (Bartholomew et al., 1941). Bartholomew et al. (1941) named this disorder in oranges as "blossom-end granulation or stylar-end granulation." Section drying (SD), primarily vesicle collapse (VC), has been known in grapefruit (Albrigo et al., 1980). This disorder resulted in drying of the affected fruit segments. Since G occurs infrequently in grapefruit (Bartholomew et al., 1941) and visible symptoms of SD are different from G, it was thought that SD in grapefruit might occur via a different mechanism.

The present study focused on comparing the physiological and biochemical changes characteristic of G and VC in grapefruit, especially regarding the structural and compositional alterations of cell wall polysaccharides.

Changes in Juice-Vesicle Components in Disordered Juice-Vesicles

G results in the swelling and hardening of affected vesicles. Bartholomew et al. (1941) and Sinclair and Jolliffe (1961) demonstrated a fresh weight increase in granulated vesicles. In agreement with their work, a 60% increase in fresh weight was observed in granulated vesicles (Table. 6). Granulated vesicles contained more dry matter but dry weight per fresh weight decreased. This observation probably indicates a decrease in tissue density as observed

Table. 6. Comparisons of juice-vesicle components between normal and disordered tissue of 'Marsh Seedless' grapefruit.

	Granulation		Vesicle Collapse	
	Normal	Granulated	Normal	Collapsed
	mg. g FW^{-1} (mg. vesicle^{-1})			
Soluble sugars	69.4[z] (6.9)	44.7 (8.1)	65.8 (5.3)	48.5 (1.3)
Acid (%)	0.9	0.7	1.2	0.7
Moisture (%)	90.2	91.6	90.2	68.4
Fresh Weight	(107.5)	(173.7)	(104.4)	(46.3)
Dry Weight	117.9 (11.9)	63.6 (14.5)	119.0 (12.4)	113.1 (5.2)
Ethanol-insoluble solids	6.2 (0.89)	13.5 (2.34)	8.8 (0.92)	20.9 (0.97)

Note: Normal vesicles were isolated from disordered fruit (VC or G).

[z] Number are the average of 3 replications. Numbers in parentheses are on a per vesicle.

in granulated oranges by Chakrawar and Singh (1977a). However, in VC there were decreases in both dry weight and fresh weight on a per vesicle basis (Table. 6). These results are consistent with the observation that VC vesicles were severely shriveled at the final stages of the disorder. Bartholomew et al. (1941) and Chakrawar and Singh (1977b) reported that granulated vesicles contained more water than normal vesicles. Bartholomew et al. (1941) suggested that water in granulated vesicles existed in a bound form. A comparison of moisture contents between disordered and normal vesicles demonstrated a slight decrease in collapsed vesicles and a slight increase in granulated vesicles (2.7%); however, the differences were not significant. The inconsistent values obtained for moisture contents in granulated vesicles were probably due to the presence of vesicles at different stages of the disorder.

The decreases in sugar and acid as total soluble solids and titratable acids, respectively, in granulated vesicles are well documented (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961; Matsumoto, 1964; Awasthi and Nauriyal, 1972; El-Zeftawi, 1973, 1976; Chakrawar and Singh, 1977b; Gilfillan and Stevenson, 1979). In the present study, granulated vesicles contained 64% of the sugar level and 80% of the acid level found in normal vesicles (Table. 6). However, granulated vesicles actually contained more total soluble sugars because of their increased size. VC also resulted in decreases in sugars and acids. Thus, collapsed

vesicles contained 74% of the sugar levels and 60% of the acid levels found in normal vesicles .

Ethanol-insoluble-solids (EIS) increased in granulated and collapsed vesicles on a per g fresh weight basis. However, on a per vesicle basis, a higher EIS content was observed for granulated vesicles whereas collapsed vesicles exhibited no change compared to normal vesicles (Table. 6). Sinclair and Jolliffe (1961) suggested that vesicles might produce more cell-wall material during the development of G. No increase in EIS in VC indicates that the physiological events occurring in granulated vesicles did not occur in collapsed vesicles. Analysis of wall polysaccharides revealed marked differances between the disorder types. When expressed on a per mg EIS basis, there were no differances in total pectin content between normal and disordered vesicles. However, a decrease in water-soluble pectins (WSP) was observed in both disorder types (Table. 7). Fifteen and 48% decreases in WSP were observed in granulated and collapsed vesicles, respectively. Differences in WSP were also found between normal vesicles; normal vesicles from vesicle collapsed fruit contained 19.1 µg of WSP·mg EIS^{-1}, whereas visually normal vesicles isolated from granulated vesicles contained 31.2 µg WSP·mg EIS^{-1}. This suggests that normal vesicles from vesicle collapsed fruit are in an early stage of VC. A decrease in WSP in granulated vesicles has been reported (Gilfillan and Stevenson, 1977; El-Zeftawi, 1978).

Table 7. Comparisons of cell-wall polysaccharides between normal and disordered vesicles of 'Marsh Seedless' grapefruit.

	Granulation		Vesicle Collapse	
	Normal	Granulated	Normal	Collapsed
	µg sugars·mg EIS^{-1} (µg sugars·vesicle^{-1})			
Pectins[z]				
TP	375.5[y] (334.2)	354.2 (828.8)	349.2 (321.3)	350.6 (340.1)
WSP	31.2 (27.8)	26.8 (62.7)	19.1 (17.6)	10.0 (9.7)
EDTA	115.8 (103.1)	82.8 (193.3)	130.4 (119.9)	134.8 (130.8)
Hemicelluloses	110.9 (98.7)	87.0 (203.5)	103.3 (95.0)	97.9 (94.2)
Celluloses	133.7 (118.9)	142.2 (332.)	120.8 (111.1)	111.1 (107.8)

Note. Normal vesicles were isolated from disordered fruit (VC and G).

[z] TP: total pectins, WSP: water-soluble pectins, EDTA: chelator-soluble pectins.

[y] Numbers are the average of 3 replications. Numbers in parentheses are on a per vesicle basis.

Chelator-soluble pectins decreased in granulated vesicles (Table. 7). The fact that total pectins did not change indicates that granulated vesicles contain more insoluble pectins. Similar difference was not found in collapsed vesicles.

Hemicellulose content decreased in granulated vesicles compared to normal vesicles on an EIS basis but was double on a per vesicle basis (Table. 7). However, changes in hemicellulose contents were not found in collapsed vesicles Cellulose contents did not changed in either disorder on an EIS basis but was nearly 3 times higher in granulated vesicles on a per vesicle basis (Table. 7). Since on a per vesicle basis, granulated vesicles contained 1.8 to 2.5 times more cell-wall components (pectins, hemicellulose, and cellulose) than normal vesicles (Table. 7), the increase in EIS in granulated vesicles was largely accounted for by an increase in wall polysaccharides. These results suggest that G may be associated with an anomalous increase in cell-wall synthesis and/or changes in cell-wall turn-over rate at the late stages of fruit development. However, the differences in cell-wall contents were not observed in VC.

Lignin components were detected histochemically by phloroglucinol-HCL in both normal and disordered vesicles (G and VC) (data not shown). Microscopic examination indicated that the increase in lignin was much pronounced in granulated vesicles compared to normal or collapsed vesicles. Lignin synthesis occurs at the primary walls and

extends inward; thus, lignified cell walls become thicker (Northcote, 1972). Bartholomew et al. (1941) reported the occurrence of lignin in granulated vesicles from 'Valencia' oranges. Also, Burns and Achor (1988) found lignin components histochemically in the walls of juice-vesicle cells, particularly in partially collapsed vesicles of grapefruit. Since Kordan (1964) observed vascular-like components with secondary wall thickening in cells of vesicle stalks in lemon, lignins might be produced in grapefruit vesicle cells besides in the stalk region. Whether or not lignification in G occurs as a result of a stress response is not known. Asada and Matsumoto (1987) reported that plant cells produced lignin when infected by fungi or when subjected to other forms of stress (e.g., injury).

Ultrogel Chromatography of Pectins Derived from Vesicles

Gel filtration may not provide totally accurate information about the molecular weight (MW) of cell-wall polymers because these polysaccharides are extremely heterogeneous and hydrogen, ionic and/or hydrophobic interaction with the gels may affect their fractionation behavior (Huber and Lee, 1986). However, this technique is still useful for ascertaining changes in the MW of cell-wall polysaccharides. This study was conducted to examine the MW characteristics of polysaccharides isolated from the cell walls of normal and disordered juice-vesicles.

The elution behevior of WSP from normal and granulated vesicles was nearly similer. Void polymers were more prevelent in WSP from granulated vesicles (Fig. 9 A and B). The elutlon patterns of WSP from normel end collapsed vesicles exhiblted marked differences (Fig. 9 C and D). There was a great decrease in the high MW polymers from collepsed vesicles whereas polymers fractionetlng on the gel increased significantly. These results can be regarded as evidance for WSP degradation in collapsed vesicles.

Chelator-soluble pectins from normal and granulated vesicles showed almost identicel elutlon patterns (Flg. 10A end B). A sllght difference was observed in excluded polymers (fraction number 20 - 30). Approximately 90% of the chelator-soluble pectins from both disordered end normel vesicle eluted in the void region. Molecular weight differences in cheiator-soiubie pectins between normal and collepsed vesicles were found; chelator-soluble pectins from collepsed vesicles were distributed evenly between high and low MW regions, whereas approximately 90% of chelator-soluble pectins from normel vesicles were eluted in the void reglon (Fig. 10C end D). Since the combined pectins in WSP end chelator-soluble pectins compriaed approxlmately 40% of the total pectins (Teble. 7), the data obtained from gel chromatography indicate that 1) granulated vesicles contelned pectins unaltered in terms of their MW, and 2) pectin degradation might be a potential fector responsible for the collapse of vesicles in VC.

Fig. 9. Ultrogel AcA 22 profile of water-soluble-pectins from normal and disordered juice-vesicles of 'Marsh Seedless' grapefruit. A, pectins from normal vesicles from granulated fruit, B; pectins from granulated vesicles, C; pectins from normal vesicles from vesicle collapsed fruit, D, pectins from collapsed vesicles.

A
B
C
D
Abs (520 nm)
Abs (520 nm)
Fraction Number
Fraction Number

Fig. 10. Ultrogel AcA 22 profile of chelator-soluble-pectins from normal and disordered vesicles of 'Marsh Seedless' grapefruit. A, pectins from normal vesicles from granulated fruit, B; pectins from granulated vesicles, C; pectins from normal vesicles from vesicle collapsed fruit, D; pectins from collapsed vesicles.

A
B
C
D
Abs (520 nm)
Abs (520 nm)
Fraction Number
Fraction Number

Polygalacturonase (endo-PG) is well known to be associated with pectin depolymerization in many fruit types (Huber, 1983a; Brady, 1986). In citrus, only exo-PG was reported in the fruit and abscision zone of leaf tissue (Riov, 1974, 1975). However, in this study, PG activity was not detected in either normal and disordered vesicles (data not shown). Although exo-PG may be present, exo-PG alone is unable to degrade polymeric pectins (Reese, 1977). An alternate explanation of pectin degradation in collapsed vesicles is non-PG-mediated modification. An example of non-PG-mediated modification of pectins can be found in muskmelone fruit (McCollum, 1987). Muskmelons contain no endo-PG but low-MW pectin appears during fruit ripening. The factor(s) causing this change has not been found. Acid hydrolysis of sucrose was found in acidic vacuoles in lime fruit (Echeverria and Burns, unpublished data). If glycosidic bonds are cleaved by strong acid, this mechanism could affect high MW cell-wall polymers. Mature grapefruit juice contained maximun 2.7% acid content (Sinclair, 1984) but the extracted juice from overmature grapefruit in this study contained 1.2% (Table. 6). Whether or not pectins can be degraded by this acid level is not known.

Ion-Exchange Chromatography of Soluble Pectins

WSP from normal and granulated vesicles exhibited somewhat similar elution behavior on ion exchange but the elution peak of WSP from granulated vesicles was observed at 200 mM NaCl, whereas it was observed at approximately 300 mM

for WSP from normal vesicles (Fig. 11A and B).

Water-soluble-pectins from normal and collapsed vesicles exhibited differences on DEAE-sephadex (Fig. 11C and D). Approximately 90% of the WSP from both normal and VC vesicles were eluted in a range of the gradient corresponding to a NaCl concentration of about 500 mM, however, the elution peak was observed at approximately 300 mM NaCl for pectins from normal vesicles and at 420 mM NaCl for pectins from collapsed vesicles. The difference in WSP elutions at 200 mM and 420 mM NaCl for granulated and collapsed vesicles, respectively, may be significant.

Chelator-soluble pectins from granulated and normal vesicles were eluted similarly on DEAE-sephadex (Fig. 12A and B). If methoxy content is higher in pectins from granulated vesicles, as reported by Sinclair and Jolliffe (1961), pectins from granulated vesicles should elute at a lower ionic-strength. However, the ion-exchange chromatography data were not consistent with this idea. The high methoxy content in granulated vesicles in the previous study (Sinclair and Jolliffe, 1961) may have originated from the increased lignin or perhaps from pectins insoluble in water or EDTA.

The elution behavior of chelator-soluble pectins from collapsed and normal vesicles showed slight difference. The elution peak of chelator-soluble pectins from normal and granulated vesicles was observed at about 400 mM NaCl, whereas it was observed at about 350 mM NaCL in G. Also,

Fig. 11 Ion-exchange profile of water-soluble-pectins from normal and disordered vesicles of 'Marsh Seedless' grapefruit. A: pectins from normal vesicles from granulated fruit, B: pectins from granulated vesicles. C: pectins from normal vesicles from vesicle collapsed fruit, D: pectins from collapsed vesicles.

A
B
C
D
Abs (320 nm)
NaCl (M)
Fraction Number

Fig. 12. Ion-exchange profile of chelator-soluble-pectins from normal and disordered vesicles of 'Marsh Seedless' grapefruit. A; pectins from normal vesicles from granulated fruit, B; pectins from granulated vesicles, C; pectins from normal vesicles from vesicle collapsed fruit, D; pectins from collapsed vesicles.

A
B
C
D
Abs (326 nm)
NaCl (M)
Fraction Number

the distribution of pectins was slightly different between normal and collapsed vesicles. In gernerel, cheletor-soluble pectin did not show definite differences in both disordere.

Ultrogel Chromatography of Hemicelluloses

Hemicelluloses extracted from normal, granuleted, and collapsed vesicles exhibited similar elution patterns on Ultrogel 34 (Fig. 13A, B, C, and D). Hemicelluloses in void fractions were increased slightly in G.

The ultrogel profiles of Fig. 13 suggest that slight hemicellulose modification may occur in both normal and disordered vesicles, but if cell-well degradation is a prime factor responsible for vesicle collapse, pectin degradation is probably of more importance.

Neutral Sugars in Soluble Pectins and Hemicelluloses

Total neutrel-suger content of WSP increased 2.5 times in VC and 1.6 times in G compared to each normal vesicles (Table. 8). There was an increase in all neutral sugars associated with WSP from VC, particularly arabinose and galactose (the 2 major sugars in WSP). However, in granulated vesicles, galactose and glucose increased significently whereas arabinose remained constant. The increase in glucose may be the result of starch metabolism in granulated vesicles because granuleted fruit have been reported to contain starch (Charkrawa and Singh, 1977b). By what mechanism neutral sugars increased in both disordered vesicles (VC and G) is not well understood. Grose (1986)

Fig. 13. Ultrogel AcA 34 profile of hemicelluloses from normal and disordered vesicles of 'Marsh Seedless' grapefruit. A: hemicelluloses from normal vesicles from granulated fruit, B: hemicelluloses from granulated vesicles, C; hemicelluloses from normal vesicles from vesicle collapsed fruit, D: hemicelluloses from collapsed vesicles.

A
B
C
D
Abs (450 nm)
Fraction Number

Table 8. Neutral sugars in soluble pectins from normal and disordered juice-vesicles of 'Marsh Seedless' grapefruit.

Neutral Sugars	Granulation		Vesicle Collapse	
	Normal	Granulated	Normal	Collapsed
	% Mol·acid sugar[1]			
	Water-soluble-pectins			
Rhamnose	0.7	3.3	4.7	10.3
Arabinose	45.8	45.0	23.3	67.5
Xylose	0.7	9.3	0.9	3.0
Mannose	nd[2]	1.2	nd	0.7
Galactose	37.1	62.4	26.3	88.9
Glucose	nd	19.5	nd	9.8
Total	55.2	180.9	84.3	140.2
	Chelator-soluble-pectins			
Rhamnose	2.9	2.3	1.7	tr
Arabinose	4.3	4.0	0.3	2.1
Xylose	0.9	nd	nd	nd
Mannose	nd	nd	nd	nd
Galactose	0.7	tr	tr	tr
Glucose	nd	nd	nd	nd
Total	8.1	6.3	2.7	2.1

Note: Neutral sugars were analyzed from pectins recovered after ion-exchange chromatography with two injections per replication.

[2]nd and tr indicate non-detectable and trace, respectively.

reported that water-soluble neutral sugars increase during ripening of tomatoes. Thus, the increase in neutral sugars in disordered tissue may be an expression of vesicle senescence.

A major sugars in hemicelluloses from normal or disordered vesicles wee xylose (Table. 9). The differences of sugar composition in hemicellulose was not detected except glucoes in granulated vesicles, hemicelluloses in granulated vesicles contained 27% morm glucose than those in normal vesicles. The above results suggest that only water-soluble pectins isolated from VC and G vesicles undergo compositional changes. Neither chelator-soluble pectins nor hemicelluloses showed definite changes in their composition when affected by vesicle disorders.

Scanning-Electron-Microscopy

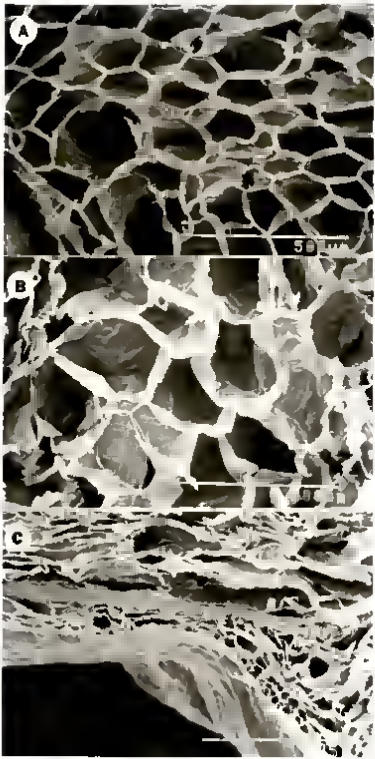
Scanning-electron-microscopy (SEM) disclosed significant differences between normal, granulated, and collapsed vesicles. The micrographs were taken from the inner juice-cells (Fig. 14) or from epidermal regions (Fig. 15). In normal vesicles, the inner juice-cells were well preserved (Fig. 14A). However, some collapsed cells were observed in granulated vesicles (Fig. 14B). In VC, almost all of the cells were partially or completely collapsed, resulting in stacked layers of cell walls (Fig. 14C). An increase in size of juice-cells but not epidermal and hypodermal cells was noticeable in granulated vesicles (Fig. 14B and 15B).

Table 9 Neutral sugars in hemicelluloses from normal and disordered juice-vesicles of 'Marsh Seedless' grapefruit.

Neutral Sugars	Granulation		Vesicle Collapse	
	Normal	Granulated	Normal	Collapsed
		% Mole		
Rhamnose	tr	tr	tr	nd
Fucose	1.7	2.9	2.4	1.6
Arabinose	10.2	7.3	11.5	7.9
Xylose	59.9	55.7	56.5	60.4
Mannose	8.0	9.9	9.0	11.1
Galactose	7.5	8.3	9.0	6.8
Glucose	12.5	15.9	11.5	12.3

Note: Neutral sugars were analysed from hemicelluloses recovered after ion-exchange chromatography with two injections per replication.

Fig. 14. Scanning electron micrographs of inner juice cells of 'Marsh Seedless' grapefruit juice-vesicles. A; normal vesicls, B; granulated vesicle, C; collapsed vssicle.


A
50 μm
B
C

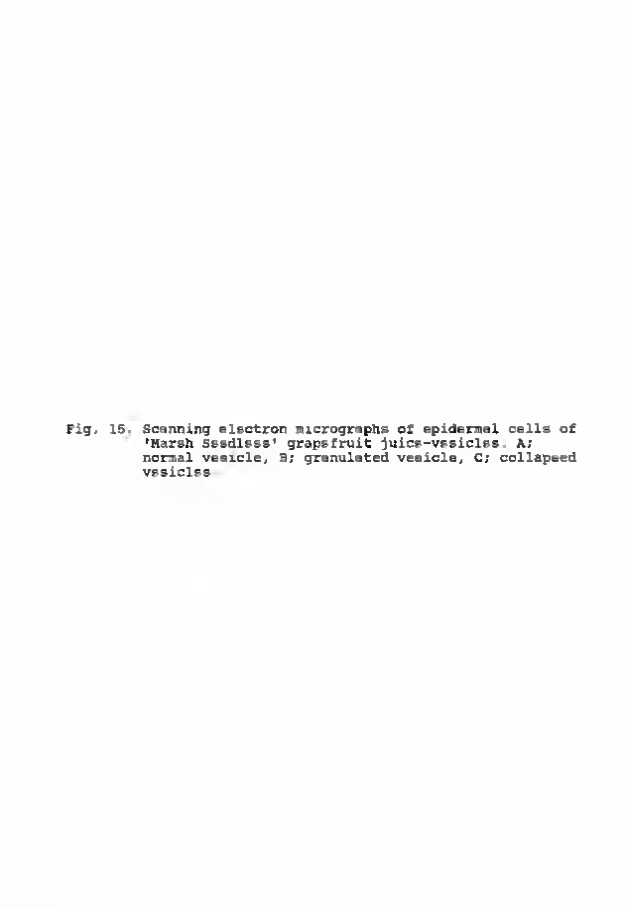
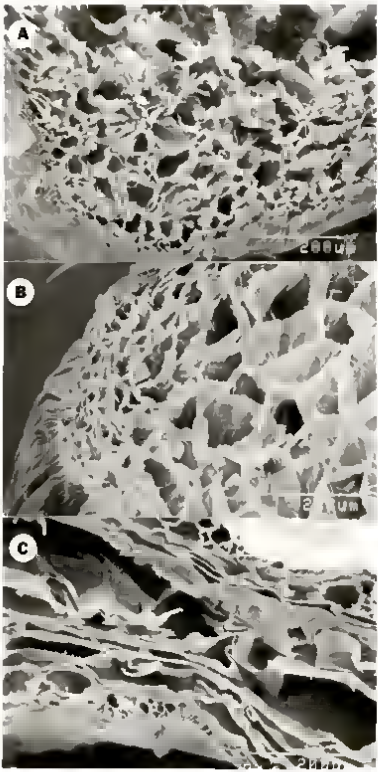


Fig. 15. Scanning electron micrographs of epidermal cells of 'Marsh Seedless' grapefruit juice-vesicles. A; normal vesicle, B; granulated vesicle, C; collapsed vesicles


A
B
2 um
C

SUMMARY AND CONCLUSIONS

Vesicle disorders result in section drying (SD) of the affected segments in grapefruit. Grapefruit produced in Florida can suffer from SD (granulation end/or vesicle collapse), particularly when harvested late in the season (May). SD can occur at any part of the fruit but the stylar- and stem-end tended to be more susceptible in these senescent fruit. After storage, the major vesicle disorder was vesicle collapse. The major symptom of this disorder is a complete collapse of the affected vesicles without any hardening.

Marked variations between years and groves were observed, indicating that various grove and climatic factors may be related to the incidence of section drying.

Crop loss also can also occur due to the in-fruit seed germination during storage when fruit are harvested late in the season. In-fruit germination was closely correlated to SD. This is probably because juice released by SD can serve as a moisture source for seed germination.

Vesicle collapse (probably associated with degradation of the cell wall) is a prime disorder in grapefruit produced in Florida. Fruit quality is reduced when affected by

vesicle collapse and/or granulation. Both disorders result in decreases in sugars and acids. Vesicle collapse differed distinctly from granulation. Granulated vesicles contained approximately 2 times more cell-wall components (pectins, hemicellulose, and cellulose) compared to normal vesicles. Vesicle collapse appeared to be associated with a modification in pectin molecular weight. This molecular weight change did not appear to be mediated by polygalacturonase. Pectin modification was not observed in granulated vesicles. Hemicellulose did not appear to be related to either vesicle disorder (VC and G).

The increase in size of juice-cells which occurred in G indicates that vesicles affected by G undergo new growth whereas juice-cells in VC vesicles partially or completely collapse.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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